70% Ethanol Fixation

This method was successful in our lab using prostate tissue and for our specific objectives. Investigators must be aware that they will need to tailor the following protocol for their own research objectives and tissue under study.

1. Materials

70% ethanol, 4°C

2. Method

1. The initial fixation of tissue specimens in 70% ethanol is similar for either subsequent low-melt polyester or paraffin embedding.

2. In the surgical suite immediately after resection, place the tissue specimen directly onto wet ice or into 70% ethanol at 4°C.

   TIP: Placement into 70% EtOH may restrict subsequent processing, e.g., inking of surgical markings.

3. Transport to the pathology department for evaluation and further processing.

4. Grossly process the tissue specimen into thin sections in order to allow quick penetration by the fixative. In general, the specimen should be a maximum of 3 mm thick. If the tissue is a large mass, it should be grossly cut into several slices and/or small pieces.

   TIP: This is particularly important for molecular profiling studies because ethanol does not penetrate tissue as rapidly as formalin and it is critical to minimize the interval between surgical removal and cellular fixation.

5. For paraffin-embedding:

   - Fix the specimen overnight in 70% ethanol at 4°C.
   - Place into a cassette and dehydrate on a standard tissue processor.
   - Eliminate the formalin step.

6. For low-melt polyester:

   - Place into a sufficiently large container with a 10X volume:volume amount of cold 70% ethanol.
   - Fix overnight.

   TIP: Small biopsy samples can be fixed for shorter time intervals, typically 4-6 hours.

Note: There are many issues to consider when evaluating new fixation and embedding schemes for molecular profiling efforts. These include the effects of the new protocol on subsequent macromolecule stability and recovery, histology, immunohistochemistry, and in-situ hybridization studies. Associated issues include the ease of use of the method and the cost of the required reagents. For more information and research examples, see Prostate Tissue Processing.